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REVIEW

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# Adenosine receptor signaling: a key to opening the blood–brain door

Margaret S. Bynoe<sup>1\*</sup>, Christophe Viret<sup>2</sup>, Angela Yan<sup>1</sup> and Do-Geun Kim<sup>1</sup>

## Abstract

The aim of this review is to outline evidence that adenosine receptor (AR) activation can modulate blood–brain barrier (BBB) permeability and the implications for disease states and drug delivery. Barriers of the central nervous system (CNS) constitute a protective and regulatory interface between the CNS and the rest of the organism. Such barriers allow for the maintenance of the homeostasis of the CNS milieu. Among them, the BBB is a highly efficient permeability barrier that separates the brain micro-environment from the circulating blood. It is made up of tight junction-connected endothelial cells with specialized transporters to selectively control the passage of nutrients required for neural homeostasis and function, while preventing the entry of neurotoxic factors. The identification of cellular and molecular mechanisms involved in the development and function of CNS barriers is required for a better understanding of CNS homeostasis in both physiological and pathological settings. It has long been recognized that the endogenous purine nucleoside adenosine is a potent modulator of a large number of neurological functions. More recently, experimental studies conducted with human/mouse brain primary endothelial cells as well as with mouse models, indicate that adenosine markedly regulates BBB permeability. Extracellular adenosine, which is efficiently generated through the catabolism of ATP via the CD39/CD73 ecto-nucleotidase axis, promotes BBB permeability by signaling through A<sub>1</sub> and A<sub>2A</sub> ARs expressed on BBB cells. In line with this hypothesis, induction of AR signaling by selective agonists efficiently augments BBB permeability in a transient manner and promotes the entry of macromolecules into the CNS. Conversely, antagonism of AR signaling blocks the entry of inflammatory cells and soluble factors into the brain. Thus, AR modulation of the BBB appears as a system susceptible to tighten as well as to permeabilize the BBB. Collectively, these findings point to AR manipulation as a pertinent avenue of research for novel strategies aiming at efficiently delivering therapeutic drugs/cells into the CNS, or at restricting the entry of inflammatory immune cells into the brain in some diseases such as multiple sclerosis.

**Keywords:** Adenosine, Ectonucleotidases, P1 purinergic receptors, CNS, Brain, Blood–brain barrier, Permeability, Endothelial cells, Drug delivery

## Background

Neurons of the central nervous system (CNS) are separated from the lumen of blood vessels by physical barriers which ensure both protective and homeostatic functions [1, 2]. The main barriers are the blood–brain barrier (BBB) and its spinal cord counterpart, the blood–spinal cord barrier. Such barriers are made of tightly connected endothelial cells that line the CNS microvasculature and form a more highly restrictive barrier than

endothelial cells of the peripheral circulation. These cells are characterized by a markedly restricted pinocytosis and transcytosis potential, the expression of dedicated transporters that regulate the influx/efflux of nutritive/toxic compounds, a low expression of leukocyte adhesion molecules and the elaboration of specialized luminal structures involved in tight and adherens junctions that efficiently restrain passive diffusion of blood-borne molecules [3–9]. The BBB endothelium is surrounded by basement membrane, pericytes and processes from neighboring astrocytes that contribute to the so-called neurovascular unit (NVU) which regulates barrier functions, homeostasis and stability [10] (Fig. 1). Astrocytes

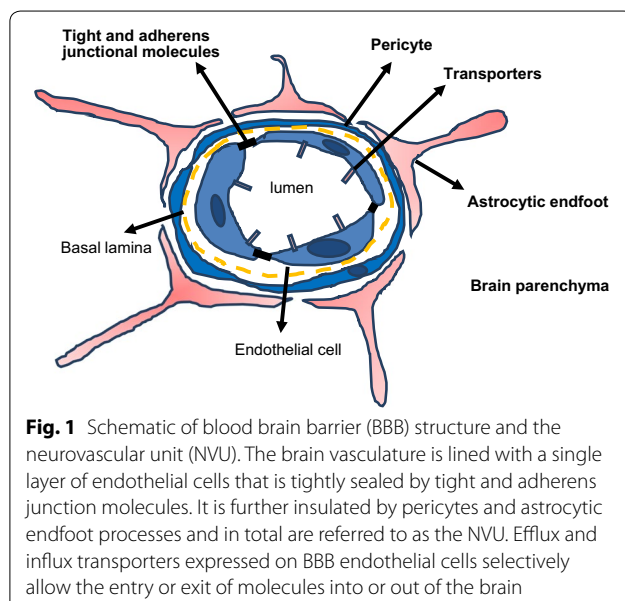
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provide nutrients that are important for endothelial cells activation/polarization; and they function as a scaffold, providing structural support for the vasculature. While astrocytic processes enwrap endothelial cells, they also interact with microglial cells and neurons [11, 12]. Astrocytes regulate BBB tightness by providing soluble factors that aid in endothelial cell proliferation and growth or are involved in maintenance of BBB integrity [13, 14]. While controlling the passage of molecules between the brain blood circulation and the brain microenvironment in the healthy brain, the BBB may also contribute to the pathogenesis of several neurological disorders such as neurodegenerative diseases, under conditions of abnormal functioning [1]. Therefore, dissecting the mechanisms underlying the properties of the BBB is necessary for understanding both the physiology of the healthy CNS as well as the development of some brain pathologies.

Adenosine is a nucleoside naturally produced by neurons and glial cells. Through a well characterized set of receptors called P1 purinergic receptors, adenosine has long been known to act as a potent modulator of various brain functions through the regulation of multiple neurotransmitters, receptors and signaling pathways [15]. Here, we review recent *in vivo* and *in vitro* studies that point to the adenosine-AR axis as an important regulatory pathway controlling BBB permeability to macromolecules and cells, and propose that manipulation of AR signaling might represent a new approach to achieve an efficient delivery of therapeutic agents into brain parenchyma.

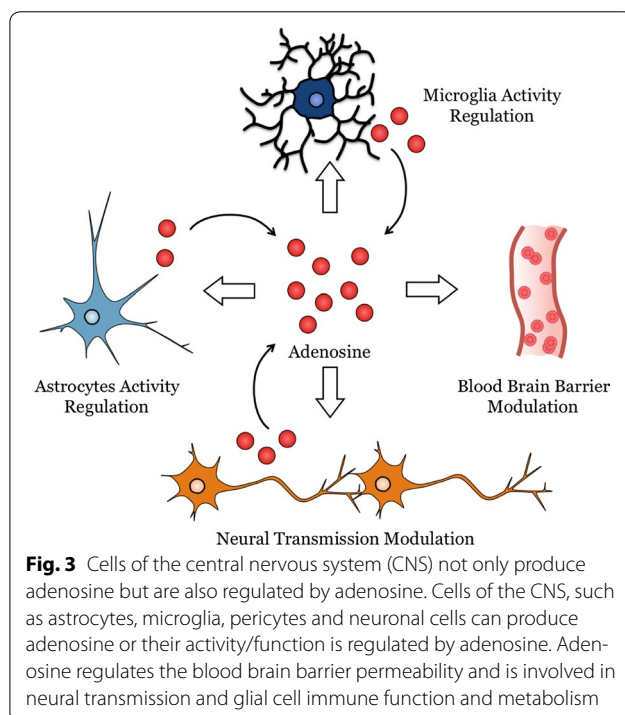
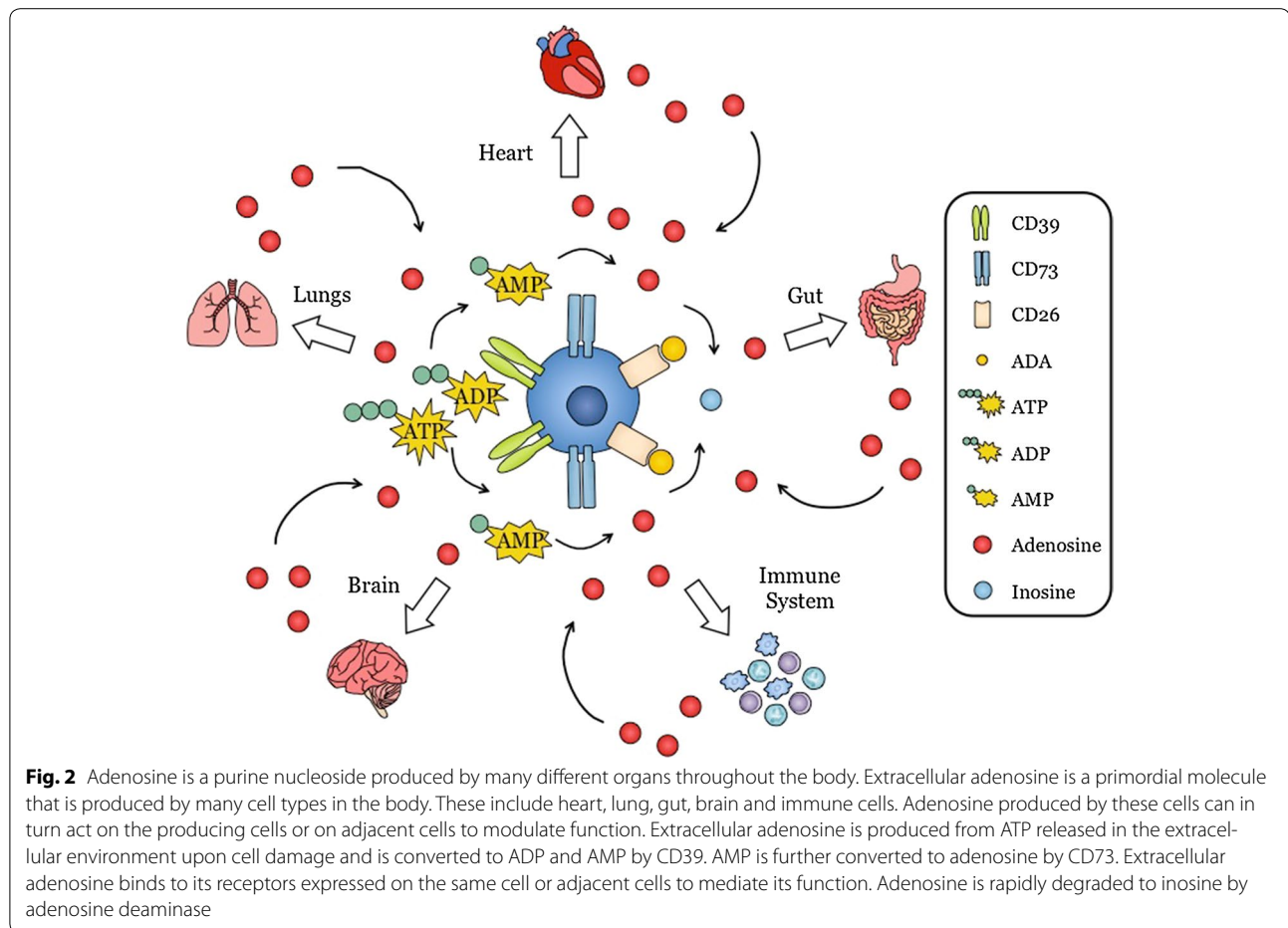


## Adenosine and ARs in CNS physiology

Adenosine is a purine nucleoside involved in a myriad host functions. It is a potent immune regulator and, in addition, is notable for its role in regulating inflammation, wound healing, angiogenesis and myocardial contractility (Fig. 2). Within the CNS, adenosine is released by both neurons and glial cells. It regulates multiple physiological functions such as sleep, arousal, neuroprotection, learning and memory, cerebral blood circulation as well as pathological phenomena such as epilepsy. These effects involve adenosine modulation of neuronal excitability, vasodilatation, release of neurotransmitters, synaptic plasticity/function and local inflammatory processes [15–17] (Fig. 3).

Within cells, adenosine is an intermediate for the synthesis of nucleic acids and adenosine triphosphate (ATP). It is generated from 5'-adenosine monophosphate (AMP) by 5'-nucleotidase and can be converted back to AMP by adenosine kinase. Adenosine can also be derived from S-adenosylhomocysteine (SAH) due to the activity of SAH hydrolase. Intracellular adenosine is metabolized into inosine by adenosine deaminase (ADA) and into AMP by adenosine kinase. Inosine formed by deamination can exit the cell intact or can be degraded to hypoxanthine, xanthine and ultimately uric acid. A low level of cellular adenosine can be quickly released in the extracellular space via equilibrative nucleoside transporters (ENTs). This release increases when intracellular adenosine concentration is augmented (ischemia, hypoxia, seizures).

Importantly, adenosine can also be directly generated outside the cell through the breakdown of cell-released adenosine tri/diphosphate (ATP/ADP) by coupled cell surface molecules with catalytically active sites (ectonucleotidases) that are abundant in the brain. The ecto-nucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1) or CD39, converts ATP/ADP into AMP and the glycosyl phosphatidylinositol(GPI)-linked ecto-5'-nucleotidase (Ecto5'NTase) or CD73, converts AMP to adenosine by promoting the hydrolysis of phosphate esterified at carbon 5' of nucleotides with no activity for 2'- and 3'-monophosphates [18, 19]. Human CD73 assembles as a dimer of GPI-anchored glycosylated mature molecules. Each monomer contains an N-terminal domain that binds divalent metal ions and a C-terminal that binds the nucleotide substrate. The ecto-nucleotidases-mediated generation of adenosine from adenine nucleotides is very rapid (about 1 ms). Adenosine half-life in the extracellular space is about 10 s. Under basal conditions, most extracellular adenosine appears to re-enter cells through equilibrative transporters. A small fraction can be irreversibly converted into inosine and its derivatives (hypoxanthine, xanthine, uric acid) by ADA



and xanthine oxidase (Fig. 2). Such a fraction increases under conditions of hypoxia/ischemia [20, 21]. Extracellular adenosine can also be targeted by ectokinases to regenerate AMP, ADP and ATP. The concentration of extracellular adenosine is maintained at low levels within the brain (ranging from 25 to 250 nM) which represent the balance between the export/generation of extracellular adenosine and its metabolism. Under pathophysiological circumstances, such as hypoxia or ischemia, extracellular adenosine concentrations can increase up to 100 fold [22, 23]. Because of its rapid metabolism, adenosine acts locally rather than systemically [24].

Extracellular adenosine exerts its action through seven-transmembrane domain, G-protein coupled receptors (GPCRs) that are connected to distinct transduction pathways. There are four different subtypes of ARs,  $A_1$ ,  $A_{2A}$ ,  $A_{2B}$  and  $A_3$  with distinct expression profiles, pharmacological characteristics and associated signaling pathways [25, 26].  $A_1$  and  $A_3$  ARs are inhibitory and suppress adenylyl-cyclase which produces cyclic-AMP (cAMP) while  $A_{2A}$  and  $A_{2B}$  ARs are stimulatory for adenylyl-cyclase [27]. In turn,  $A_{2B}$ -induced cAMP can upregulate

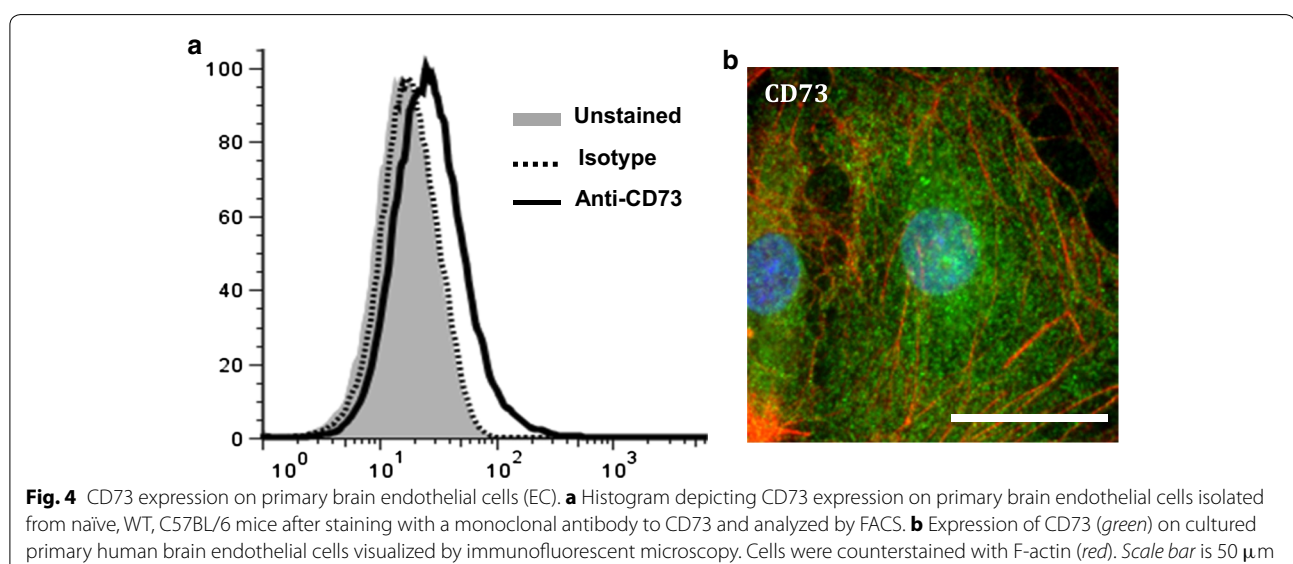
CD73 [28].  $A_1$  and  $A_{2A}$  ARs have high affinity for adenosine (about 70 and 150 nM respectively) whereas  $A_{2B}$  and  $A_3$  have a markedly lower affinity for adenosine (about 5100 and 6500 nM, respectively) [27]. This suggests that  $A_1$  and  $A_{2A}$  may be the major ARs that are activated by physiological levels of extracellular adenosine within the CNS. Accordingly, unlike  $A_1$  and  $A_{2A}$  receptors,  $A_{2B}$  receptor engagement in the brain is triggered by higher adenosine levels such as levels associated with cell stress or tissue damage [25]. The expression level of ARs varies depending on the type of cells or organs where they are expressed [22].

The influence of adenosine in the CNS depends both on its local concentration and on the expression level of ARs. The  $A_1$  receptor is highly expressed in the brain cortex, hippocampus, cerebellum and in spinal cord [25, 29, 30] and at lower levels at other sites of the brain [31]. In multiple sclerosis (MS) patients, the  $A_1$  receptor expression level appears to be decreased in CD45 positive glial cells of the brain [32]. The  $A_{2A}$  receptor expression is high in the olfactory tubercle, dorsal and ventral striatum and throughout the choroid plexus which forms the blood-cerebrospinal fluid (CSF) barrier [33–36] and more moderate in the meninges, cortex and hippocampus [29, 33, 36]. The steady state expression of  $A_{2A}$  receptor permits for example the proper regulation of extracellular glutamate titer by adenosine, through modulation of glutamate release and control of glutamate transporter-1-mediated glutamate uptake [37–39].  $A_{2A}$  receptors interact negatively with D2 dopamine receptors [40].  $A_{2A}$  receptor expression in glial cells such as astrocytes is substantially upregulated by stress factors including pressure, pro-inflammatory factors (interleukin (IL)-1 $\beta$ ,

tumor necrosis factor (TNF)- $\alpha$ ) or hypoxia. In contrast to  $A_1$  and  $A_{2A}$ ,  $A_{2B}$  and  $A_3$  receptors are expressed at relatively low levels within brain [31].

### Expression of the CD73 ecto-enzyme on CNS barrier cells

Some studies have pointed to CD73 as a regulator of tissue barrier function [41]. Within the CNS, ATP can be released from neurons or other cells such as astrocytes. As mentioned above, CD39 catalyses the conversion of proinflammatory ATP/ADP into AMP and CD73 subsequently converts AMP into adenosine [42]. Thus, the proper functioning of CD39/CD73 ectonucleotidases concomitantly ensures the production of extracellular adenosine and the extinction of purinergic P2 receptor-dependent, ATP-induced signaling due to reduction of the ATP/ADP pool. Both of these effects contribute to the anti-inflammatory potential of the CD39/CD73 axis. Along with colon and kidney, the brain has particularly high levels of CD73 enzyme activity [41]. Similar to the  $A_{2A}$  receptor, CD73 shows its strongest expression level in the CNS within the choroid plexus epithelium and is also detected on glial cells of the submeningeal areas of the spinal cord [22, 27, 43]. CD73 can be expressed on many types of endothelial cells [44]. Its expression on BBB endothelial cells remains low under steady state conditions relative to peripheral endothelial cells (Fig. 4a). It is present on mouse (Bend.3) and human (hCMEC/D3) brain endothelial cell lines in vitro [27, 45]. Unlike human brain endothelial cells [46, 47], CD73 expression on primary mouse brain endothelial cells was very low and not detected in vivo [43] (Fig. 4a). However, CD73 expression can be detected in primary human brain endothelial





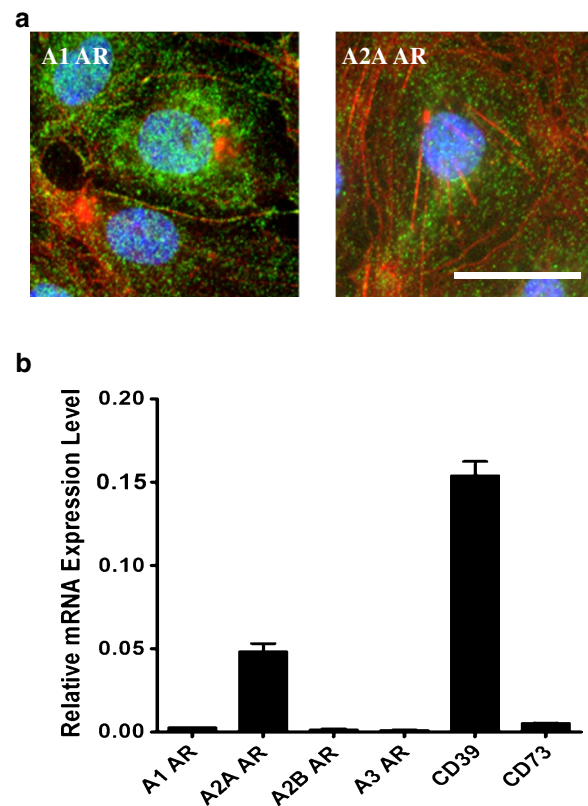
cells (Fig. 4b) [45, 48]. CD73 expression is sensitive to cyclic AMP (cAMP) and hypoxia-inducible factor (HIF)1 through its promoter [49]. Interferon (IFN)- $\beta$  increases CD73 expression and adenosine concentration at the level of the CNS microvasculature, BBB and astrocytes [46, 47] and through enhanced adenosine production, may contribute to the anti-inflammatory effect of IFN- $\beta$  in MS treatment.

### AR signaling in the NVU

Functional  $A_1$ ,  $A_{2A}$ ,  $A_{2B}$  and  $A_3$  receptors are all expressed at moderate levels in glial cells under physiological context [50] and this level is upregulated under inflammatory conditions or brain injury. All P1 purinergic receptors appear to be present on cultured oligodendrocytes [51], on microglial cells [42] and are functional on astrocytes [52–56] (Fig. 3). In astrocytes, ARs engagement is not only important for glutamate uptake regulation ( $A_{2A}$  receptor) but also to maintain cellular integrity ( $A_1$  receptor) [53, 57, 58], protect from hypoxia-related cell death ( $A_3$  receptor) [57] and regulate CCL2 chemokine production ( $A_3$  receptor) [59]. In microglial cells,  $A_{2A}$  receptor engagement inhibits process extension and migration while  $A_1$  and  $A_3$  receptors engagement have the opposite effect [42].  $A_1$  and  $A_{2A}$  receptor transcripts are detectable in Z310 epithelial cells derived from mouse choroid plexus [43]. As to CNS endothelial cells,  $A_1$ ,  $A_{2A}$  and  $A_{2B}$  receptor transcripts and proteins were expressed in hCMEC/D3 human brain endothelial cells [45]. Also,  $A_1$  and  $A_{2A}$  ARs are expressed in primary human brain endothelial cells (Fig. 5a). In Bend.3 mouse brain endothelial cells, transcripts and proteins for  $A_1$  and  $A_{2A}$  receptors were detected [27]. Finally, both  $A_1$  and  $A_{2A}$  receptor proteins were found expressed in primary mouse brain endothelial cells and transcripts and proteins for both  $A_1$  and  $A_{2A}$  receptors were present in brain endothelial cells in mice [27] (Fig. 5b).

### AR signaling and CNS barrier permeability

The recent notion that adenosine could play a substantial regulatory role in CNS barrier permeability stems from the observation that extracellularly generated adenosine positively regulates the entry of lymphocytes into the brain and spinal cord during disease development in the experimental autoimmune encephalomyelitis (EAE) model [43] and the observation that irradiated  $A_{2A}$  AR deficient mice reconstituted with wild-type bone marrow cells developed only very mild signs of EAE with virtually no CD4<sup>+</sup> T cell infiltration in spinal cord [43]. In line with an important role for AR signaling in regulating the permeability of the BBB is the observation that inhibition of ARs by caffeine (a broad-spectrum AR antagonist) prevents the alteration of BBB function induced by



**Fig. 5** Expression of adenosine receptors (ARs) and adenosine-generating enzymes on brain endothelial cells. **a**  $A_1$  and  $A_{2A}$  ARs expression green on primary human brain endothelial cells by immunofluorescence assay (IFA). Cells were counterstained with F-actin (red). **b** Relative mRNA expression level of ARs, CD39 and CD73 by quantitative PCR (q-PCR) in mouse primary brain endothelial cells. Scale bar is 50  $\mu$ m

cholesterol or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in animal models of neurodegenerative diseases [60, 61].

Recent observations support the notion that engagement of ARs on brain endothelial cells modulates BBB permeability in vivo. Experimental recruitment of ARs either by the broad spectrum agonist NECA or the engagement of both  $A_1$  and  $A_{2A}$  receptors by selective agonists (CCPA and CGS21680) cumulatively and transiently augmented BBB permeability facilitating the entry of intravenously infused macromolecules (including immunoglobulins such as the anti- $\beta$ -amyloid 6E10 antibody) into the CNS [27]. Accordingly, the analysis of engineered mice lacking these receptors reveals a limited entry of macromolecules into the brain upon exposure to AR agonists. CNS entry of intravenously delivered macromolecules was also induced by the FDA-approved,  $A_{2A}$  AR agonist Lexiscan: 10 kDa dextran was detectable within the CNS of mice as soon as 5 min after drug

injection. The maximum increase in BBB permeability was observed at about 30 min after injection both in mice and rats. The limited half-life of Lexiscan (about 3 min) is likely to account for the lower duration of BBB permeability relative to that induced by NECA (half-life: 5 h).

Upon exposure to NECA or Lexiscan, monolayers of Bend3 mouse brain endothelial cells (CD73<sup>+</sup>, A<sub>1</sub> AR<sup>+</sup>, A<sub>2A</sub> AR<sup>+</sup>) lowered their transendothelial cell electrical resistance, a phenomenon known to be associated with increased paracellular space and augmented permeability [62, 63]. AR activation by agonists was indeed associated with augmented actinomyosin stress fiber formation indicating that ARs signaling initiates changes in cytoskeletal organization and cell shape. These processes are reversed as the half-life of the AR agonist decreases. At the level of tight junctions, signaling induced by A<sub>1</sub> and A<sub>2A</sub> receptor agonists altered the expression level of tight junction proteins such as claudin-5 and ZO-1, and particularly of occludin in cultured brain endothelial cells [27]. The exact signaling circuits connecting AR engagement and cytoskeletal remodeling remain to be dissected. In agreement with these findings and with the observation that human brain endothelial cells do respond to adenosine in vitro, agonist-induced A<sub>2A</sub> receptor signaling transiently permeabilized a primary human brain endothelial cell monolayer to the passage of both drugs and Jurkat human T cells in vitro [48]. Interestingly, transendothelial migration of Jurkat cells was primarily of the paracellular type. The permeabilization process involved RhoA signaling-dependent morphological changes in actin-cytoskeletal organization, a reduced phosphorylation of factors involved in focal adhesion (namely Ezrin-Radixin-Moesin (ERM) and focal adhesion kinase (FAK)) as well as a marked downregulation of both claudin-5 and vascular endothelial (VE)-cadherin [48], two factors instrumental for the integrity of endothelial barriers. Hence, by regulating the expression level of factors crucially involved in tight junction integrity/function, signaling induced through receptors for adenosine acts as a potent, endogenous modulator of BBB permeability in mouse models as well as in human cellular models in vitro.

Some G proteins such as G<sub>α</sub> subunits can influence the activity of small GTPases RhoA and Rac1 that are known modulators of cytoskeletal organization. RhoA and Rac1 are responsive to adenosine signaling and promote actin cytoskeleton remodeling [62, 64–66]. The precise molecular events linking A<sub>1</sub>/A<sub>2A</sub> AR engagement to changes in the expression pattern of factors involved in tight junction functioning remains however to be analyzed in detail. In particular, whether both canonical (G protein-dependent) and non-canonical (e.g. G protein-independent, β-arrestin-related) signaling pathways contribute to such regulation is an open question. Another interesting

issue relates to the capacity of A<sub>1</sub> and A<sub>2A</sub> receptors to form heterodimers [67] and the possible impact of such oligomeric receptors on the regulation of CNS barrier by ARs agonists.

### CD73 and AR signalling in immune cell entry into the CNS

Besides its capacity to regulate the local inflammatory context through consumption of ATP and generation of adenosine, the expression of CD39/CD73 by endothelial cells can regulate homeostasis by preventing high local concentrations of ATP that promote thrombosis and generating adenosine which instead, contributes to an antithrombotic microenvironment [44]. The CD39/CD73 axis also regulates leukocyte migration induced by chemokines [68, 69] and immune cell adhesion to endothelial cells. Such adhesion is favored by high ATP concentrations and limited by adenosine, with mutant mice lacking CD39 or CD73 having augmented level of leukocyte adhesion to endothelial cells [70, 71]. Thus, adenosine contributes to restraining leukocyte recruitment and platelet aggregation and might be important to control vascular inflammation.

We have observed that CD73-generated adenosine promotes the entry of inflammatory lymphocytes into the CNS during EAE development [43]. Genetically manipulated mice unable to generate extracellular adenosine due to deficiency in the ectonucleotidase CD73 (CD73<sup>-/-</sup>) are resistant to lymphocyte entry into the CNS and EAE development relative to wild type animals and such a phenotype could be recapitulated in regular mice by using either the broad spectrum AR antagonist, caffeine or the SCH58261 that selectively antagonizes the signaling induced by adenosine-bound A<sub>2A</sub> receptor [43, 72–74]. This effect was remarkable since auto-reactive lymphocytes from CD73<sup>-/-</sup> mice indeed harbor an enhanced inflammatory potential. In addition, the expression of CD73 (and presumably its enzymatic activity) on either T cells or CNS cells was sufficient to support lymphocyte entry into the CNS since CD4 T cells from wild type donors (i.e. CD73<sup>+</sup>) could mediate a milder yet substantial level of EAE pathogenesis in CD73<sup>-/-</sup> recipient mice [43].

Since CD73 and the A<sub>1</sub>/A<sub>2A</sub> receptors are expressed at the level of the choroid plexus, locally produced extracellular adenosine is likely to act in an autocrine manner. Given that A<sub>1</sub> and A<sub>2A</sub> receptor recruitment are functionally opposed to each other and harbor some differences in their affinity for adenosine [26], the regional extracellular concentration of adenosine may strongly influence the response of neighboring cells expressing both receptors. A<sub>1</sub> receptor signaling may be involved at low adenosine concentrations while A<sub>2A</sub> receptor signaling is likely to

become prominent at elevated adenosine concentrations. Thus, CD73 enzymatic activity at the choroid plexus and the regional adenosine levels are likely to influence local inflammatory events. Interestingly, the choroid plexus is suspected to represent a primary entry site for immune cells during neuroinflammation [3–5] and for steady state immunosurveillance [6, 75]. By combining the gene expression pattern of chemokines and chemokine receptors relevant to EAE in CD73 null mutant versus control mice developing EAE and the effect of the broad spectrum AR agonist NECA on the expression profile of these molecules in unmanipulated animals, it was possible to identify CX3CL1/fractalkine, a chemokine/adhesion molecule [76], as the major factor induced by extracellular adenosine in the brain of mice developing EAE [77]. The cleavage of the cell surface-expressed form of CX3CL1 by ADAM-10 and -17 factors generates a local CX3CL1 gradient [78]. The selective  $A_{2A}$  AR agonist CGS21680 caused an increase in CX3CL1 level in the brain of treated mice. Conversely, the  $A_{2A}$  AR antagonist SCH58261 protected mice from CNS lymphocyte infiltration and EAE induction recapitulating the phenotype of CD73 null mutant mice. Thus, the augmented CX3CL1 expression level seen in the brain of EAE developing mice can be regulated by  $A_{2A}$  AR signaling. During EAE, the greatest increase in CX3CL1 occurred at the choroid plexus and returned to normal when mice recovered from disease. As choroid plexus cells express both CD73 and  $A_{2A}$  AR, they have the intrinsic capacity to generate and respond to extracellular adenosine. In vitro,  $A_{2A}$  AR engagement on the choroid plexus epithelial cell line CPLacZ-2 by CGS21680 induced CX3CL1 expression and promoted lymphocyte transmigration suggesting that CX3CL1 induction by extracellular adenosine contributes to lymphocyte migration into the brain parenchyma during EAE. In agreement with an important role for CX3CL1 in EAE pathogenesis is the fact that CX3CL1 blockade by neutralizing antibodies prevented lymphocyte entry into the CNS and EAE development [77]. This notion is in line with the elevated serum level of CX3CL1 which can be observed during CNS inflammation including MS patient brain lesions [79–81]. Importantly, there was a positive correlation between CX3CL1 expression levels and the relative frequency of lymphocytes present in the CSF of inflamed brains [80, 82]. Moreover, relative to BBB endothelial cells, choroid plexus epithelial cells constitutively express high levels of CD73. Blockade of CD73 or  $A_{2A}$  AR inhibits inflammatory cells entry into the CNS [43, 77]. Thus, at the level of the choroid plexus, induction of  $A_{2A}$  receptor signaling by elevated local adenosine concentrations is likely to contribute to immune cell entry into the brain parenchyma.

Among immune cells, the CX3CL1 receptor (CX3CR1) was detected on a sizable fraction of CD4 T cells, CD8 T cells, macrophages and NK cells in mice [33]. CNS CX3CL1 might also modulate neuroinflammation by recruiting a subset of CNS resident NK cells able to attenuate the aggressiveness of autoreactive CD4 T cells of the Th17 effector type [83, 84]. Interestingly, while the frequency of inflammatory immune cells is significantly decreased in the CNS of  $A_{2A}$  AR<sup>-/-</sup>, CD73<sup>-/-</sup> or mice treated with  $A_{2A}$  AR antagonist, the numbers/frequency of CD4<sup>+</sup>CD25<sup>+</sup> T regulatory cells in these mice were similar to WT. This suggests that CD73/ $A_{2A}$  AR signaling may preferentially regulate inflammatory immune cells entry into the CNS but confers less stringency on these suppressor T cells.

### Perspectives for improved delivery of therapeutic factors within the CNS

Although the BBB serves a protective role, it can constitute a complication for treatment in CNS diseases by hindering the entry of therapeutic compounds into the brain [85]. Researchers have focused on uncovering ways to manipulate the BBB to promote access to the CNS [86]. Determining how to safely and effectively do this could impact the treatment of various neurological diseases, ranging from neurodegenerative disorders to brain tumors. This implies simultaneous treatments with agents susceptible to increase permeation of CNS barriers. Current approaches involve barrier disruption which is induced by drugs such as mannitol or Cereport/RMP-7. Hypertonic mannitol, is active through shrinking of endothelial cells [87, 88] but can cause epileptic seizures and does not allow for repeated use [89, 90]. The bradykinin analog (Cereport/RMP-7) has shown some potential in transiently increasing normal BBB permeability [91] but did not give satisfactory results in clinical trial [92] despite some efficacy in treating rodent models of CNS pathologies [93–96].

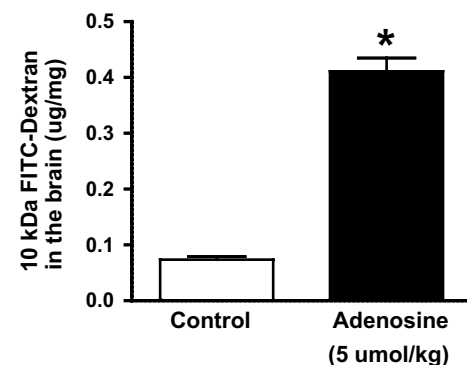
The barrier permeability can also be circumvented, for instance by direct injection of drugs into ventricles [87, 97]. More recent approaches involve the delivery of drugs during compression waves induced by high-intensity focused ultrasounds [98]. Both these approaches are invasive and may lead to permanent brain damage. Another strategy involves chemical modifications of compounds in order to confer upon them some capacity to cross CNS barriers. For example, increasing the lipophilicity of drugs can enhance their capacity to cross the BBB although it often requires an increase in their size which limits the cell penetration capacity [99]. Alternatively, therapeutic compounds can be linked to factors that trigger receptor-mediated endocytosis. Coupling a compound to an antibody directed to the transferrin receptor



can promote delivery of proteins to the brain in rats [100, 101]. However, the endocytic activity of endothelial cells is rather limited at the BBB and the expression level of the relevant receptor needs to be sufficient.

For adenosine to exert biological effect, CD73 and ARs must be present on the same cell or on adjacent cells, because adenosine acts locally due to its short half-life. CD73, A<sub>1</sub> and A<sub>2A</sub> ARs are indeed expressed on BBB endothelial cells in mice and humans. While CD73 is highly and constitutively expressed on choroid plexus epithelial cells that form the blood to CSF barrier, its expression on brain endothelial barrier cells is low under steady state conditions, but increases in neuroinflammatory diseases or under conditions where adenosine is produced in response to cell stress/inflammation or tissue damage. In mice, pharmacological activation or inhibition of the A<sub>2A</sub> AR expressed on BBB cells opens and tightens the BBB, respectively, to entry of macromolecules or cells. The observation that adenosine can modulate BBB permeability upon A<sub>2A</sub> receptor activation suggest that this pathway might represent a valuable strategy for modulating BBB permeability and promote drug delivery within the CNS [27, 48]. Factors such as the FDA-approved, A<sub>2A</sub> AR agonist Lexiscan, or a broad-spectrum agonist, NECA, increased BBB permeability and supported macromolecule delivery to the CNS in experimental setting [27]. Such exogenous agonists might represent a new avenue of research for therapeutic macromolecule delivery to the human CNS. Of note is the fact that the window of the induced permeability correlated with the half-life of the agonist. Thus, BBB permeation induced by NECA treatment (half-life, 4 h) lasted significantly longer than that induced by Lexiscan treatment (half-life, 2.5 min) [27]. Interestingly, despite its short half-life, extracellular adenosine permeabilized the BBB to entry of 10 kDa dextran (Fig. 6). Approaches based on the use of such an agonist might be useful for the delivery of therapeutic antibodies to the CNS since invasive delivery is a commonly used method [102] and is not patient-friendly.

Further studies are needed to better understand the mechanisms involved in BBB permeability modulation by A<sub>1</sub>/A<sub>2A</sub> receptors-triggered signaling as well as the parameters susceptible to optimize the timing of such a modulation (Fig. 7). In particular, in vitro cell based model of BBB where cerebral endothelial cells are co-cultured with other components of the NVU such as pericytes or astrocytes (co-cultures and triple co-cultures systems) [103] should be considered for evaluation. Another important issue relates to the question of the identification of the CNS areas where the microvasculature is significantly permeabilized by A<sub>1</sub>/A<sub>2A</sub> receptor-induced signaling and more generally whether or



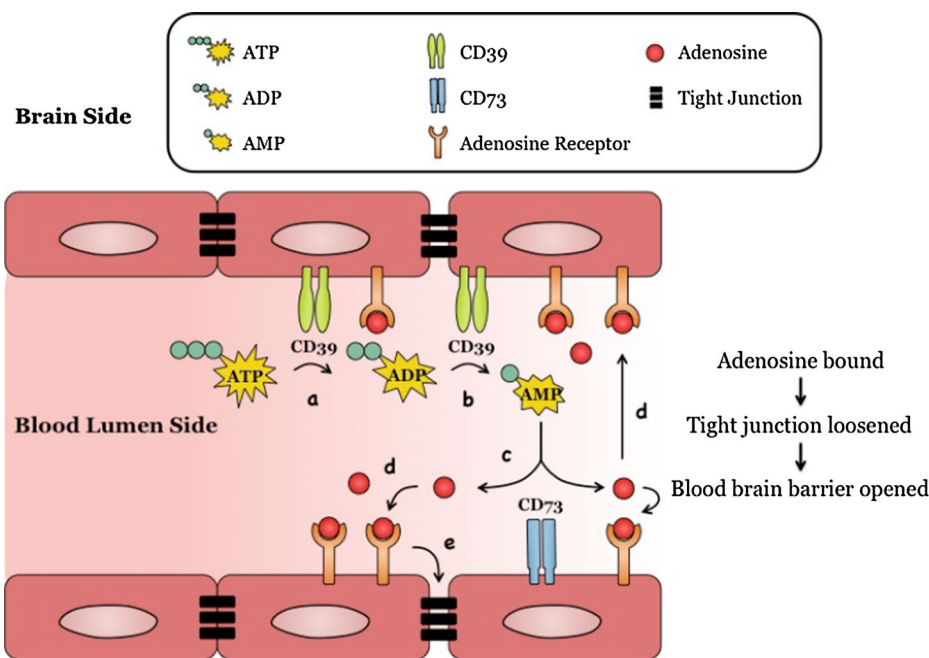
**Fig. 6** Adenosine increases the permeability of the blood brain barrier to 10 kDa FITC dextran. Concomitant administration of Adenosine and 10 kDa FITC-Dextran in C57BL/6 mice with exogenous adenosine induces significantly higher accumulation of FITC-Dextran into the brain than PBS control treatment group ( $n = 2$ , asterisk indicates  $p < 0.01$ )

not, there exists a restricted or a global permeabilization within the CNS. An alternative strategy to be explored is the experimental manipulation of the regional level of endogenous adenosine or of the responsiveness/expression level of A<sub>1</sub>/A<sub>2A</sub> receptors. Such knowledge will be instrumental in designing novel approaches for the improved delivery of drugs, therapeutic monoclonal antibodies and possibly, stem cells, within the CNS.

### Concluding remarks

Inhibiting AR signaling on BBB cells restricts the entry of macromolecules and inflammatory immune cells into the CNS with limited impact on anti-inflammatory, T regulatory cells. Conversely, activation of ARs on BBB cells promotes entry of small molecules and macromolecules in the CNS in a time-dependent manner. The duration of BBB permeabilization depends on the half-life of the AR activating agent or agonist, suggesting that AR modulation of the BBB is a tunable system. We conclude that: (1) The adenosine-based control of the BBB is an endogenous mechanism able to regulate cells and molecules entry into the CNS in basal conditions and during response to CNS stress or injury. (2) AR-induced opening of the BBB is time-dependent and reversible. (3) A tight regulation of CD73 expression on BBB cells is crucial to restrict and regulate adenosine bioavailability and prevent promiscuous BBB permeability.

Consequently, the control of BBB permeability via modulation of AR signaling is pertinent for research on the delivery of therapeutics to the CNS: (1) AR signaling is an endogenous mechanism for BBB control. (2) It has the potential for precise time-dependent control of BBB permeability. (3) Change in BBB permeability



**Fig. 7** A model: Adenosine modulation of blood brain barrier (BBB) permeability. Endothelial cells lining the brain vasculature express adenosine receptors (ARs) and CD39 and CD73. In the presence of cell stress/inflammation or tissue damage (a), ATP is released and is rapidly converted to ADP and AMP by CD39 (b) and AMP is converted to adenosine by CD73. c Adenosine binds to its receptor/s ( $A_1$  or  $A_{2A}$ ) on BBB endothelial cells (d), the activation of which induces reorganization of actin cytoskeleton in BBB endothelial cells, resulting in tight and adherens junction disassembly (e), increasing paracellular permeability

is reversible. (4) ARs are accessible directly on BBB endothelial cells. (5) Over 50 commercial reagents targeting ARs are available, with some approved by the FDA for clinical use. (6) In vivo and in vitro model systems can help to gain molecular mechanistic understanding of how adenosine naturally regulates changes in BBB permeability. Therapies aimed at treating neuro-inflammatory diseases such as MS, where inflammatory cells penetration of the CNS causes irreparable damage to CNS tissue, would ideally include one that could inhibit the entry of inflammatory immune cells into the CNS parenchyma. Many other diseases associated with CNS inflammation such as meningitis, encephalitis, and cerebritis could all benefit from inhibiting immune cell entry into the CNS. The challenge is determining how to safely and effectively do this. We hypothesize that manipulating the adenosine-ARs axis on CNS barrier cells may represent an efficient way to modulate the entry of immune cells into the CNS and to limit CNS inflammation and pathology.

#### Abbreviations

ATP: adenosine tri-phosphate; AR: adenosine receptor; BBB: blood-brain barrier; BM: basement membrane; cAMP: cyclic adenosine monophosphate; CNS: central nervous system; CSF: cerebrospinal fluid; EAE: experimental

autoimmune encephalomyelitis; Ecto5'NTase: ecto-5'-nucleotidase (CD73); E-NTPDase1: Ecto-nucleoside triphosphate diphosphohydrolase 1 (CD39); ERM: Ezrin-Radixin-Moesin; FAK: focal adhesion kinase; GPCR: G-protein coupled receptor; IL: interleukin; IFN: interferon; JAM: junction adhesion molecules; MS: multiple sclerosis; NVU: neurovascular unit; PDGF: Platelet derived growth factor; TGF: transforming growth factor; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor; VE-cadherin: vascular endothelial-cadherin; VSMC: vascular smooth muscle cells; ZO: zona occludens.

#### Authors' contributions

Margaret Bynoe and Christophe Viret wrote the manuscript and Angela Yan and Do-Geun Kim contributed figures and assisted with editing of the manuscript. All authors read and approved the final manuscript.

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#### Compliance with ethical guidelines

#### Competing interests

The authors declare that they have no conflict of interest.

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# References

- Saunders NR, Ek CJ, Habgood MD, Dziegielewska KM. Barriers in the brain: a renaissance? *Trends Neurosci.* 2008;31(6):279–86. doi:10.1016/j.tins.2008.03.003.
- Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron.* 2008;57(2):178–201. doi:10.1016/j.neuron.2008.01.003.
- Brown DA, Sawchenko PE. Time course and distribution of inflammatory and neurodegenerative events suggest structural bases for the pathogenesis of experimental autoimmune encephalomyelitis. *J Comp Neurol.* 2007;502(2):236–60. doi:10.1002/cne.21307.
- Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, et al. C–C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat Immunol.* 2009;10(5):514–23. doi:10.1038/ni.1716.
- Engelhardt B, Wolburg-Buchholz K, Wolburg H. Involvement of the choroid plexus in central nervous system inflammation. *Microsc Res Tech.* 2001;52(1):112–29. doi:10.1002/1097-0029(20010101)52:1<112::AID-JEMT13>3.0.CO;2-5.
- Ousman SS, Kubes P. Immune surveillance in the central nervous system. *Nat Neurosci.* 2012;15(8):1096–101. doi:10.1038/nn.3161.
- Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci.* 2006;7(1):41–53. doi:10.1038/nrn1824.
- Rubin LL, Staddon JM. The cell biology of the blood-brain barrier. *Annu Rev Neurosci.* 1999;22:11–28. doi:10.1146/annurev.neuro.22.1.11.
- Aijaz S, Balda MS, Matter K. Tight junctions: molecular architecture and function. *Int Rev Cytol.* 2006;248:261–98. doi:10.1016/S0074-7696(06)48005-0.
- Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. *Nat Med.* 2013;19(12):1584–96. doi:10.1038/nm.3407.
- Ricci G, Volpi L, Pasquali L, Petrozzi L, Siciliano G. Astrocyte-neuron interactions in neurological disorders. *J Biol Phys.* 2009;35(4):317–36. doi:10.1007/s10867-009-9157-9.
- Shih AY, Fernandes HB, Choi FY, Kozoriz MG, Liu Y, Li P, et al. Policing the police: astrocytes modulate microglial activation. *J Neurosci Off J Soc Neurosci.* 2006;26(15):3887–8. doi:10.1523/JNEUROSCI.0936-06.2006.
- Armulik A, Genove G, Mae M, Nisancioglu MH, Wallgard E, Niaudet C, et al. Pericytes regulate the blood-brain barrier. *Nature.* 2010;468(7323):557–61. doi:10.1038/nature09522.
- Abbott NJ. Astrocyte-endothelial interactions and blood-brain barrier permeability. *J Anat.* 2002;200(6):629–38.
- Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. *Annu Rev Neurosci.* 2001;24:31–55. doi:10.1146/annurev.neuro.24.1.31.
- Sebastiao AM, Ribeiro JA. Adenosine receptors and the central nervous system. *Handb Exp Pharmacol.* 2009;193:471–534. doi:10.1007/978-3-540-89615-9\_16.
- Stone TW, Ceruti S, Abbracchio MP. Adenosine receptors and neurological disease: neuroprotection and neurodegeneration. *Handb Exp Pharmacol.* 2009;193:535–87. doi:10.1007/978-3-540-89615-9\_17.
- Yegutkin GG. Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. *Biochim Biophys Acta.* 2008;1783(5):673–94. doi:10.1016/j.bbamcr.2008.01.024.
- Zimmermann H. 5'-Nucleotidase: molecular structure and functional aspects. *Biochem J.* 1992;285(Pt 2):345–65.
- Barankiewicz J, Danks AM, Abushanab E, Makings L, Wiemann T, Wallis RA, et al. Regulation of adenosine concentration and cytoprotective effects of novel reversible adenosine deaminase inhibitors. *J Pharmacol Exp Ther.* 1997;283(3):1230–8.
- Lloyd HG, Fredholm BB. Involvement of adenosine deaminase and adenosine kinase in regulating extracellular adenosine concentration in rat hippocampal slices. *Neurochem Int.* 1995;26(4):387–95 (019701869400144 J [pii]).
- Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. *Nat Rev Drug Discovery.* 2006;5(3):247–64. doi:10.1038/nrd1983.
- Wei CJ, Li W, Chen JF. Normal and abnormal functions of adenosine receptors in the central nervous system revealed by genetic knockout studies. *Biochim Biophys Acta.* 2011;1808(5):1358–79. doi:10.1016/j.bbame.2010.12.018.
- Hasko G, Linden J, Cronstein B, Pacher P. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nat Rev Drug Discovery.* 2008;7(9):759–70. doi:10.1038/nrd2638.
- Fredholm BB, Ilzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev.* 2001;53(4):527–52.
- Fredholm BB, Ilzerman AP, Jacobson KA, Linden J, Muller CE. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors—an update. *Pharmacol Rev.* 2011;63(1):1–34. doi:10.1124/pr.110.003285.
- Carman AJ, Mills JH, Krenz A, Kim DG, Bynoe MS. Adenosine receptor signaling modulates permeability of the blood-brain barrier. *J Neurosci.* 2011;31(37):13272–80. doi:10.1523/JNEUROSCI.3337-11.2011.
- Eltzschig HK, Sitkovsky MV, Robson SC. Purinergic signaling during inflammation. *N Engl J Med.* 2012;367(24):2322–33. doi:10.1056/NEJMr1205750.
- Dixon AK, Gubitz AK, Sirinathsinghji DJ, Richardson PJ, Freeman TC. Tissue distribution of adenosine receptor mRNAs in the rat. *Br J Pharmacol.* 1996;118(6):1461–8.
- Mahan LC, McVittie LD, Smyk-Randall EM, Nakata H, Monsma FJ Jr, Gerfen CR, et al. Cloning and expression of an A1 adenosine receptor from rat brain. *Mol Pharmacol.* 1991;40(1):1–7.
- Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W. Structure and function of adenosine receptors and their genes. *Naunyn-Schmiedeberg's Archiv Pharmacol.* 2000;362(4–5):364–74.
- Johnston JB, Silva C, Gonzalez G, Holden J, Warren KG, Metz LM, et al. Diminished adenosine A1 receptor expression on macrophages in brain and blood of patients with multiple sclerosis. *Ann Neurol.* 2001;49(5):650–8.
- Mills JH, Kim DG, Krenz A, Chen JF, Bynoe MS. A2A adenosine receptor signaling in lymphocytes and the central nervous system regulates inflammation during experimental autoimmune encephalomyelitis. *J Immunol.* 2012;188(11):5713–22. doi:10.4049/jimmunol.1200545.
- Rosin DL, Robeva A, Woodard RL, Guyenet PG, Linden J. Immunohistochemical localization of adenosine A2A receptors in the rat central nervous system. *J Comp Neurol.* 1998;401(2):163–86.
- Schiffmann SN, Libert F, Vassart G, Vanderhaeghen JJ. Distribution of adenosine A2 receptor mRNA in the human brain. *Neurosci Lett.* 1991;130(2):177–81.
- Svenningsson P, Hall H, Sedvall G, Fredholm BB. Distribution of adenosine receptors in the postmortem human brain: an extended autoradiographic study. *Synapse.* 1997;27(4):322–35. doi:10.1002/(SICI)1098-2396(199712)27:4<322::AID-SYN6>3.0.CO;2-E.
- Li XX, Nomura T, Aihara H, Nishizaki T. Adenosine enhances glial glutamate efflux via A2a adenosine receptors. *Life Sci.* 2001;68(12):1343–50.
- Matos M, Augusto E, Agostinho P, Cunha RA, Chen JF. Antagonistic interaction between adenosine A2A receptors and Na<sup>+</sup>/K<sup>+</sup>-ATPase-α2 controlling glutamate uptake in astrocytes. *J Neurosci Off J Soc Neurosci.* 2013;33(47):18492–502. doi:10.1523/JNEUROSCI.1828-13.2013.
- Matos M, Augusto E, Santos-Rodrigues AD, Schwarzschild MA, Chen JF, Cunha RA, et al. Adenosine A2A receptors modulate glutamate uptake in cultured astrocytes and gliosomes. *Glia.* 2012;60(5):702–16. doi:10.1002/glia.22290.
- Fredholm BB, Svenningsson P. Adenosine-dopamine interactions: development of a concept and some comments on therapeutic possibilities. *Neurology.* 2003;61(11 Suppl 6):S5–9.
- Colgan SP, Eltzschig HK, Eckle T, Thompson LF. Physiological roles for ecto-5'-nucleotidase (CD73). *Purinergic Signal.* 2006;2(2):351–60. doi:10.1007/s11302-005-5302-5.
- Burnstock G, Boeynaems JM. Purinergic signalling and immune cells. *Purinergic Signal.* 2014;10(4):529–64. doi:10.1007/s11302-014-9427-2.
- Mills JH, Thompson LF, Mueller C, Waickman AT, Jalkanen S, Niemela J, et al. CD73 is required for efficient entry of lymphocytes into the central nervous system during experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA.* 2008;105(27):9325–30. doi:10.1073/pnas.0711175105.
- Koszalka P, Ozuyaman B, Huo Y, Zernecke A, Fogel U, Braun N, et al. Targeted disruption of cd73/ecto-5'-nucleotidase alters thromboregulation and augments vascular inflammatory response. *Circ Res.* 2004;95(8):814–21. doi:10.1161/01.RES.0000144796.82787.6f.

45. Mills JH, Alabanza L, Weksler BB, Couraud PO, Romero IA, Bynoe MS. Human brain endothelial cells are responsive to adenosine receptor activation. *Purinergic Signalling*. 2011;7(2):265–73. doi:[10.1007/s11302-011-9222-2](#).
46. Niemela J, Ifergan I, Yegutkin GG, Jalkanen S, Prat A, Airas L. IFN-beta regulates CD73 and adenosine expression at the blood-brain barrier. *Eur J Immunol*. 2008;38(10):2718–26. doi:[10.1002/eji.200838437](#).
47. Airas L, Niemela J, Yegutkin G, Jalkanen S. Mechanism of action of IFN-beta in the treatment of multiple sclerosis: a special reference to CD73 and adenosine. *Ann N Y Acad Sci*. 2007;1110:641–8. doi:[10.1196/annals.1423.067](#).
48. Kim DG, Bynoe MS. A2A Adenosine Receptor Regulates the Human Blood-Brain Barrier Permeability. *Mol Neurobiol*. 2014; doi:[10.1007/s12035-014-8879-2](#).
49. Narravula S, Lennon PF, Mueller BU, Colgan SP. Regulation of endothelial CD73 by adenosine: paracrine pathway for enhanced endothelial barrier function. *J Immunol*. 2000;165(9):5262–8.
50. Hasko G, Pacher P, Vizi ES, Illes P. Adenosine receptor signaling in the brain immune system. *Trends Pharmacol Sci*. 2005;26(10):511–6. doi:[10.1016/j.tips.2005.08.004](#).
51. Stevens B, Porta S, Haak LL, Gallo V, Fields RD. Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. *Neuron*. 2002;36(5):855–68.
52. Dare E, Schulte G, Karovic O, Hammarberg C, Fredholm BB. Modulation of glial cell functions by adenosine receptors. *Physiol Behav*. 2007;92(1–2):15–20. doi:[10.1016/j.physbeh.2007.05.031](#).
53. Ciccarelli R, Ballerini P, Sabatino G, Rathbone MP, D'Onofrio M, Caciagli F, et al. Involvement of astrocytes in purine-mediated reparative processes in the brain. *Int J Develop Neurosci Off J Int Soc Develop Neurosci*. 2001;19(4):395–414.
54. Fields RD, Burnstock G. Purinergic signalling in neuron-glia interactions. *Nat Rev Neurosci*. 2006;7(6):423–36. doi:[10.1038/nrn1928](#).
55. Biber K, Klotz KN, Berger M, Gebicke-Harter PJ, van Calcar D. Adenosine A1 receptor-mediated activation of phospholipase C in cultured astrocytes depends on the level of receptor expression. *J Neurosci Off J Soc Neurosci*. 1997;17(13):4956–64.
56. Mahamed DA, Mills JH, Egan CE, Denkers EY, Bynoe MS. CD73-generated adenosine facilitates *Toxoplasma gondii* differentiation to long-lived tissue cysts in the central nervous system. *Proc Natl Acad Sci USA*. 2012;109(40):16312–7. doi:[10.1073/pnas.1205589109](#).
57. Bjorklund E, Styrislav B, Anskjaer GG, Hansen M, Halling-Sorensen B. Dichlobenil and 2,6-dichlorobenzamide (BAM) in the environment: what are the risks to humans and biota? *Sci Total Environ*. 2011;409(19):3732–9. doi:[10.1016/j.scitotenv.2011.06.004](#).
58. D'Alimonte I, Ballerini P, Nargi E, Buccella S, Giuliani P, Di Iorio P, et al. Staurosporine-induced apoptosis in astrocytes is prevented by A1 adenosine receptor activation. *Neurosci Lett*. 2007;418(1):66–71. doi:[10.1016/j.neulet.2007.02.061](#).
59. Wittendorp MC, Boddeke HW, Biber K. Adenosine A3 receptor-induced CCL2 synthesis in cultured mouse astrocytes. *Glia*. 2004;46(4):410–8. doi:[10.1002/glia.20016](#).
60. Chen X, Gawryluk JW, Wagener JF, Ghribi O, Geiger JD. Caffeine blocks disruption of blood brain barrier in a rabbit model of Alzheimer's disease. *J Neuroinflamm*. 2008;5:12. doi:[10.1186/1742-2094-5-12](#).
61. Chen X, Lan X, Roche I, Liu R, Geiger JD. Caffeine protects against MPTP-induced blood-brain barrier dysfunction in mouse striatum. *J Neurochem*. 2008;107(4):1147–57. doi:[10.1111/j.1471-4159.2008.05697.x](#).
62. Wojciak-Stothard B, Potempa S, Eichholtz T, Ridley AJ. Rho and Rac but not Cdc42 regulate endothelial cell permeability. *J Cell Sci*. 2001;114(Pt 7):1343–55.
63. Dewi BE, Takasaki T, Kurane I. In vitro assessment of human endothelial cell permeability: effects of inflammatory cytokines and dengue virus infection. *J Virol Methods*. 2004;121(2):171–80. doi:[10.1016/j.jviromet.2004.06.013](#).
64. Jou TS, Schneeberger EE, Nelson WJ. Structural and functional regulation of tight junctions by RhoA and Rac1 small GTPases. *J Cell Biol*. 1998;142(1):101–15.
65. Schreiber G, Kooij G, Reijkerker A, van Doorn R, Gringhuis SI, van der Pol S, et al. Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, PI3 kinase, and PKB signaling. *FASEB J Off Publ Feder Am Soc Exper Biol*. 2007;21(13):3666–76. doi:[10.1096/fj.07-8329com](#).
66. Sohail MA, Hashmi AZ, Hakim W, Watanabe A, Zipprich A, Groszmann RJ, et al. Adenosine induces loss of actin stress fibers and inhibits contraction in hepatic stellate cells via Rho inhibition. *Hepatology*. 2009;49(1):185–94. doi:[10.1002/hep.22589](#).
67. Sheth S, Brito R, Mukherjee D, Rybak LP, Ramkumar V. Adenosine receptors: expression, function and regulation. *Int J Mol Sci*. 2014;15(2):2024–52. doi:[10.3390/ijms15022024](#).
68. Linden J. Cell biology. Purinergic chemotaxis. *Science*. 2006;314(5806):1689–90. doi:[10.1126/science.1137190](#).
69. Salmi M, Jalkanen S. Cell-surface enzymes in control of leukocyte trafficking. *Nat Rev Immunol*. 2005;5(10):760–71. doi:[10.1038/nri1705](#).
70. Petrovic-Djergovic D, Hyman MC, Ray JJ, Bouis D, Visovatti SH, Hayasaki T, et al. Tissue-resident ecto-5' nucleotidase (CD73) regulates leukocyte trafficking in the ischemic brain. *J Immunol*. 2012;188(5):2387–98. doi:[10.4049/jimmunol.1003671](#).
71. Takedachi M, Qu D, Ebisuno Y, Oohara H, Joachims ML, McGee ST, et al. CD73-generated adenosine restricts lymphocyte migration into draining lymph nodes. *J Immunol*. 2008;180(9):6288–96.
72. Thompson LF, Takedachi M, Ebisuno Y, Tanaka T, Miyasaka M, Mills JH, et al. Regulation of leukocyte migration across endothelial barriers by Ecto-5'-nucleotidase-generated adenosine. *Nucleosides Nucleotides Nucleic Acids*. 2008;27(6):755–60. doi:[10.1080/15257770802145678](#).
73. Tsutsui S, Schnemann J, Noorbakhsh F, Henry S, Yong VW, Winston BW, et al. A1 adenosine receptor upregulation and activation attenuates neuroinflammation and demyelination in a model of multiple sclerosis. *J Neurosci Off J Soc Neurosci*. 2004;24(6):1521–9. doi:[10.1523/JNEUROSCI.4271-03.2004](#).
74. Chen GQ, Chen YY, Wang XS, Wu SZ, Yang HM, Xu HQ, et al. Chronic caffeine treatment attenuates experimental autoimmune encephalomyelitis induced by guinea pig spinal cord homogenates in Wistar rats. *Brain Res*. 2010;1309:116–25. doi:[10.1016/j.brainres.2009.10.054](#).
75. Schwartz M, Baruch K. The resolution of neuroinflammation in neurodegeneration: leukocyte recruitment via the choroid plexus. *EMBO J*. 2014;33(1):7–22. doi:[10.1002/embj.201386609](#).
76. Imai T, Hieshima K, Haskell C, Baba M, Nagira M, Nishimura M, et al. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell*. 1997;91(4):521–30.
77. Mills JH, Alabanza LM, Mahamed DA, Bynoe MS. Extracellular adenosine signaling induces CX3CL1 expression in the brain to promote experimental autoimmune encephalomyelitis. *J Neuroinflamm*. 2012;9:193. doi:[10.1186/1742-2094-9-193](#).
78. Schwarz N, Pruessmeyer J, Hess FM, Dreyer D, Pantaler E, Koelsch A, et al. Requirements for leukocyte transmigration via the transmembrane chemokine CX3CL1. *Cell Mol Life Sci CMLS*. 2010;67(24):4233–48. doi:[10.1007/s00018-010-0433-4](#).
79. Sunnemark D, Eltayeb S, Nilsson M, Wallstrom E, Lassmann H, Olsson T, et al. CX3CL1 (fractalkine) and CX3CR1 expression in myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis: kinetics and cellular origin. *J Neuroinflamm*. 2005;2:17. doi:[10.1186/1742-2094-2-17](#).
80. Kastenbauer S, Koedel U, Wick M, Kieseier BC, Hartung HP, Pfister HW. CSF and serum levels of soluble fractalkine (CX3CL1) in inflammatory diseases of the nervous system. *J Neuroimmunol*. 2003;137(1–2):210–7.
81. Broux B, Pannemans K, Zhang X, Markovic-Plese S, Broekmans T, Eijnde BO, et al. CX(3)CR1 drives cytotoxic CD4(+)CD28(–) T cells into the brain of multiple sclerosis patients. *J Autoimmun*. 2012;38(1):10–9. doi:[10.1016/j.jaut.2011.11.006](#).
82. Rancan M, Bye N, Otto VI, Trentz O, Kossmann T, Frentzel S, et al. The chemokine fractalkine in patients with severe traumatic brain injury and a mouse model of closed head injury. *J Cerebral Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab*. 2004;24(10):1110–8. doi:[10.1097/01.WCB.0000133470.91843.72](#).
83. Huang D, Shi FD, Jung S, Pien GC, Wang J, Salazar-Mather TP, et al. The neuronal chemokine CX3CL1/fractalkine selectively recruits NK cells that modify experimental autoimmune encephalomyelitis within the central nervous system. *FASEB J Off Publ Feder Am Soc Exper Biol*. 2006;20(7):896–905. doi:[10.1096/fj.05-5465com](#).



84. Hao J, Liu R, Piao W, Zhou Q, Vollmer TL, Campagnolo DI, et al. Central nervous system (CNS)-resident natural killer cells suppress Th17 responses and CNS autoimmune pathology. *J Exp Med*. 2010;207(9):1907–21. doi:[10.1084/jem.20092749](https://doi.org/10.1084/jem.20092749).
85. Hossain S, Akaike T, Chowdhury EH. Current approaches for drug delivery to central nervous system. *Curr Drug Deliv*. 2010;7(5):389–97.
86. Rajadhyaksha M, Boyden T, Liras J, El-Kattan A, Brodfuehrer J. Current advances in Delivery of biotherapeutics across the blood-brain barrier. *Curr Drug Discov Technol*. 2011;8(2):87–101.
87. Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. *NeuroRx J Am Soc Exp NeuroTherap*. 2005;2(1):3–14. doi:[10.1602/neuroRx.2.1.3](https://doi.org/10.1602/neuroRx.2.1.3).
88. Neuwelt EA, Frenkel EP, Diehl JT, Maravilla KR, Vu LH, Clark WK, et al. Osmotic blood-brain barrier disruption: a new means of increasing chemotherapeutic agent delivery. *Trans Am Neurol Assoc*. 1979;104:256–60.
89. Neuwelt EA, Specht HD, Howieson J, Haines JE, Bennett MJ, Hill SA, et al. Osmotic blood-brain barrier modification: clinical documentation by enhanced CT scanning and/or radionuclide brain scanning. *AJR Am J Roentgenol*. 1983;141(4):829–35. doi:[10.2214/ajr.141.4.829](https://doi.org/10.2214/ajr.141.4.829).
90. Marchi N, Angelov L, Masaryk T, Fazio V, Granata T, Hernandez N, et al. Seizure-promoting effect of blood-brain barrier disruption. *Epilepsia*. 2007;48(4):732–42. doi:[10.1111/j.1528-1167.2007.00988.x](https://doi.org/10.1111/j.1528-1167.2007.00988.x).
91. Borlongan CV, Emerich DF. Facilitation of drug entry into the CNS via transient permeation of blood brain barrier: laboratory and preliminary clinical evidence from bradykinin receptor agonist Cereport. *Brain Res Bull*. 2003;60(3):297–306.
92. Prados MD, Schold SJS, Fine HA, Jaeckle K, Hochberg F, Mechtler L, et al. A randomized, double-blind, placebo-controlled, phase 2 study of RMP-7 in combination with carboplatin administered intravenously for the treatment of recurrent malignant glioma. *Neuro Oncol*. 2003;5(2):96–103.
93. Bartus RT, Elliott PJ, Dean RL, Hayward NJ, Nagle TL, Huff MR, et al. Controlled modulation of BBB permeability using the bradykinin agonist, RMP-7. *Exp Neurol*. 1996;142(1):14–28. doi:[10.1006/exnr.1996.0175](https://doi.org/10.1006/exnr.1996.0175).
94. Matsukado K, Inamura T, Nakano S, Fukui M, Bartus RT, Black KL. Enhanced tumor uptake of carboplatin and survival in glioma-bearing rats by intracarotid infusion of bradykinin analog, RMP-7. *Neurosurgery*. 1996;39(1):125–33 (**discussion 33–4**).
95. Emerich DF, Snodgrass P, Dean R, Agostino M, Hasler B, Pink M, et al. Enhanced delivery of carboplatin into brain tumours with intravenous Cereport (RMP-7): dramatic differences and insight gained from dosing parameters. *Br J Cancer*. 1999;80(7):964–70. doi:[10.1038/sj.bjc.6690450](https://doi.org/10.1038/sj.bjc.6690450).
96. Elliott PJ, Hayward NJ, Dean RL, Blunt DG, Bartus RT. Intravenous RMP-7 selectively increases uptake of carboplatin into rat brain tumors. *Cancer Res*. 1996;56(17):3998–4005.
97. Cook AM, Mieure KD, Owen RD, Pesaturo AB, Hatton J. Intracerebroventricular administration of drugs. *Pharmacotherapy*. 2009;29(7):832–45. doi:[10.1592/phco.29.7.832](https://doi.org/10.1592/phco.29.7.832).
98. Bradley WG Jr. MR-guided focused ultrasound: a potentially disruptive technology. *J Am Coll Radiol*. 2009;6(7):510–3. doi:[10.1016/j.jacr.2009.01.004](https://doi.org/10.1016/j.jacr.2009.01.004).
99. Witt KA, Gillespie TJ, Huber JD, Egleton RD, Davis TP. Peptide drug modifications to enhance bioavailability and blood-brain barrier permeability. *Peptides*. 2001;22(12):2329–43. **S019697810100537X [pii]**.
100. Granholm AC, Backman C, Bloom F, Ebendal T, Gerhardt GA, Hoffer B, et al. NGF and anti-transferrin receptor antibody conjugate: short and long-term effects on survival of cholinergic neurons in intraocular septal transplants. *J Pharmacol Exp Therap*. 1994;268(1):448–59.
101. Yu YJ, Atwal JK, Zhang Y, Tong RK, Wildsmith KR, Tan C, et al. Therapeutic bispecific antibodies cross the blood-brain barrier in nonhuman primates. *Sci Transl Med*. 2014;6(261):261ra154. doi:[10.1126/scitranslmed.3009835](https://doi.org/10.1126/scitranslmed.3009835).
102. Thakker DR, Weatherspoon MR, Harrison J, Keene TE, Lane DS, Kaemmerer WF, et al. Intracerebroventricular amyloid-beta antibodies reduce cerebral amyloid angiopathy and associated micro-hemorrhages in aged Tg2576 mice. *Proc Natl Acad Sci USA*. 2009;106(11):4501–6. doi:[10.1073/pnas.0813404106](https://doi.org/10.1073/pnas.0813404106).
103. Bicker J, Alves G, Fortuna A, Falcao A. Blood-brain barrier models and their relevance for a successful development of CNS drug delivery systems: a review. *Eur J Pharm Biopharm*. 2014;87(3):409–32. doi:[10.1016/j.ejpb.2014.03.012](https://doi.org/10.1016/j.ejpb.2014.03.012).

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